ABSTRACT

Lectins are multivalent carbohydrate binding proteins of non-immune origin The richest sources of lectins are the seeds of leguminous plants, but they are found in all classes and families of organisms In recent years, they have attracted considerable attention on account of their ability to specifically recognize carbohydrates at the cell surface The introductory chapter of the thesis contains a brief description of the structural information available on lectins The rest of the thesis is concerned with structural studies on Jacalin, a lectin isolated from the seeds of jack fruit (*Artocarpus integrifolia*)

Jacalin has a molecular weight of 66,000 ± 1,200 and it recognizes, like pea nut agglutinin, T-antigen represented by the disaccharide Galβ 1-3 GalNAc The earlier work in this laboratory on Jacalin resulted in six crystal forms of which a hexagonal form (space group $P6_122$, a=129 6, b=129 6 and c=157 9 Å) was used by the author for further structural studies using the multiple isomorphous replacement (MIR) technique To this end, four heavy atom derivatives, one uranium, one europium and two platinum, were prepared by controlled soaking experiments Diffraction data from the native and the derivative crystals were collected on a Siemens-Nicolet area detector system mounted on a GX-20 Marconi Avionics rotating anode x-ray generator The raw data were processed using the XENGEN package The heavy atom positions were determined using difference Patterson and Fourier maps and the direct methods program MULTAN The native Patterson map and the heavy atom positions indicated the presence of a non-crystallographic twofold axis parallel to c The program PHARE in the CCP4 package was used for refinement of heavy

atom parameters and calculation of phase angles

To start with an MIR map and a molecular envelope were calculated at 5 5 Å resolution The protein and the solvent regions could be clearly demarcated in the map, indicating that the structure solution was proceeding in the right direction The MIR map at 3 0 Å, calculated subsequently, showed extensive features which could be interpreted in terms of secondary structural elements, but the connectivity between these elements was poor Exhaustive calculations performed to improve the quality of the MIR map using density modification procedures like solvent flattening and symmetry averaging resulted in improved maps From these density modified maps most of the Jacalin sequence could be traced without any ambiguity and the model was fitted using FRODO running on an IRIS-4D work station The structure was refined using the program package X-plor The refined model consists of 4480 out of 4740 non-hydrogen atoms accounting for 94 5% of the structure The R-factor for the refined structure is 21 2% for 12,647 reflections with $I > 2\sigma$ in the 10-3 0 Å resolution range The root mean square deviation from the ideal values in bond lengths is 0 011 Å

Each subunit of Jacalin consists of three four-stranded antiparallel β-sheets which are arranged like the faces of a triangular prism This fold has been classified as β-prism fold and it is observed for the first time in a lectin Two of the antiparallel β-sheets form Greek keys with a topology of 1,1,-3 and the third sheet has a Greek key like topology with a break between the heavy (α) and light (β) chains in the subunit because of post-translational modification The subunits possess an internal three-fold symmetry which is not well reflected in its primary structure The molecule of Jacalin is a tetramer with 222 (D_2) symmetry The crystal asymmetric

unit consists of two halves of the molecule from two independent tetramers, which are related by the non-crystallographic twofold symmetry

The author has been also involved in another lectin project in the laboratory, that on winged bean (*Psophocarpus tetragonolobus*) agglutinin The preliminary results obtained in this project are given in an appendix

A part of the results described in this thesis have been reported in the following publication

Crystallization and Preliminary X-ray Studies of the Basic Lectin from Winged Bean (*Psophocarpus tetragonolobus*)
Sankaranarayanan, R , Puri, K D , Ganesh, V , Banerjee, R , Surolia, A and Vijayan, M (1993) *J Mol Biol* **229**, 558-560